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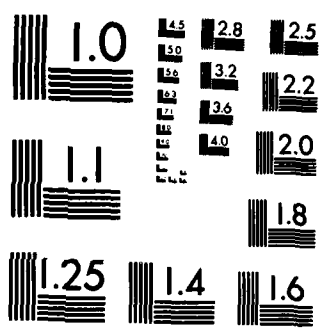

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THE EFFECTS OF COHERENT AND INCOHERENT LIGHT ON OCULAR TISSUES AND VISUAL FUNCTION IN NON-HUMAN SUBJECTS

Annual Progress Report

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)* During the period of 1 October 1980 to 30 September 1981 one micron and thin sections from additional blocks of tissue from both eyes of animal N-403 & N-239 were submitted to this contractor for sectioning and interpretation. These showed no artefact-free evidence of the effects of coherent light upon the retinas nor were ultrastructural differences detected between the treated and untreated eyes (for further details see pages 1,2, & 3 of report #1 dated Oct.1980).

Blocks of retina tissue were submitted to the contractor from both eyes of animal LA-1. Designation of which eye was exposed to coherent

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light and which eye was not, was withheld from the contractor until the contractor completed sectioning and interpretation of the submitted tissues. The animal did not show electrophysiological evidence of diminished spectral sensitivity in the treated eye. Fixation and embedding artefact was less than in blocks obtained from animals N-403 and N-239. No ultrastructural evidence of tissue alteration attributable to coherent light was found and no differences were detected between sections prepared from comparable areas of each retina.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

Report

This is a report of an investigation performed at the Eye Pathology Laboratory of the Pacific Medical Center (PMC) as a joint effort with personnel of the Division of Biorheology at the Letterman Army Institute of Research (LAIR). The study is designed to evaluate the functional and ultrastructural effects in the retinas of non-human subjects produced by chronic repeated exposure to low-levels of coherent and incoherent light.

Background

For the past decade an interdisciplinary group now located at LAIR has explored the effects of coherent and incoherent light on ocular tissue and visual function in non-human subjects in order to provide a base upon which safety standards may be established. Mechanical, thermal and photochemical retinal damage is known to be produced by exposure to optical sources which produce a discrete image on the retina of wave lengths between 400 and 1400 nm (Footnote #1). The type of damage produced depends upon the power level and exposure time. Emphasis in the present study is directed toward evaluating the effects upon the retina of chronic or repeated low-level laser exposure of the diffuse rather than discrete type. Functional alterations in the form of electro retinographic spectral sensitivity depression have been detected following exposure to diffusely applied coherent light at a level of 20 microwatts per square centimeter for a total of 20 accumulated hours over a period of three weeks. (Footnote #2).

During the period 1 October 1979 to 30 September 1980 details of Laser exposure tissue sampling techniques, ultrastructural analysis and functional animal testing were discussed, and blocks of tissue from control non-exposed animals were fixed and embedded by LAIR personnel to standardize tissue preparation techniques. Appropriate blocks were submitted to the contractor for similar standardization of sectioning techniques.

Footnote:#1 Ham, WT; Mueller, HA; Ruffolo, JJ; and Clark, AM: Photochemistry and Photobiology. Vol.29, pages 735-743, 1979.

Footnote:#2 Zwick, H; Beatrice ES; Garcia, TA: Low Level Coherent Light Effects on Rhesus - Long Term and Progressive Changes. Color Vision Deficiencies. Vol.5, 1980.

Footnote:#3 Zwick, H; Beatrice ES: Long-Term Changes in Spectral Sensitivity After Low Level Laser (514 nm) Exposure. Modern Problems in Ophthalmology.Vol.19,pp 319-325, 1978.

The eyes of three Rhesus monkeys (animals N-515, N-403, N-239) were exposed at LAIR to diffusely applied coherent light. The animals were then sacrificed, the eyes enucleated, areas of the retina and choroid excised, fixed and embedded in plastic and the blocks of tissue submitted to the contractor. (For details, please see pages 1,2, and 3 of report #1 dated October 1980). Ultrastructural studies of the tissues submitted to the contractor did not reveal fine structural evidence of alterations attributable to exposure to coherent light at the initial intensity (20 micro watts per cm/2) and duration (20 accumulated hours), or at increased intensity (40 micro watts/ $cm/^2$) and duration (40 accumulated hours).

Scope of Work

During the period 1 October 1980 to 30 September 1981 additional 1 micron and thin sections were prepared by the contractor from several additional blocks of tissue from the retina and choroid of both eyes of animals N-403 and N-239 provided to the contractor by LAIR. Labeled photographs of ultrastructural details of these tissues in the form of four loose-leaf books were submitted to J. Ryan Neville, SGRD-ULZ-RCM Building 1110, Presidio of San Francisco, California 94129. The first page of each book contains a copy of the orientation diagram provided to this contractor by personnel at Letterman Army Institute of Research designating the areas from which the blocks given to the contractor were obtained. The following pages contain photographs taken of sections prepared from each of these blocks. Each photograph is labeled with the number of the animal, the block, the eye (left or right), location of the block (e.g. temporal and inferior to macula), and of the original magnification of the photograph.

Blocks of tissue from both eyes of Rhesus LA-1 were submitted to the contractor by LAIR personnel. Details of this animal's exposure to coherent light were purposely not provided to the contractor in order to preclude prejudgement in assessing the electron microscopic sections. Specifically, information was not provided regarding which eye was exposed to coherent light, and which was not. Two loose-leaf bound books containing photographs of electron microscopic sections prepared from all blocks provided to the contractor were submitted to J. Ryan Neville, SGRD-ULZ-RCM, at Letterman Army Institute of Research.

At a conference held with LAIR personnel, contractor was requested to prepare sections through normal turtle retina for the purpose of obtaining baseline studies of the electron microscopic appearance of the retina. These studies are currently in progress.

Discussion of the Results

A review of sections prepared from all blocks of tissue submitted from animals N-403 and N-239 do not demonstrate ultrastrucural evidence of tissue alterations attributable to exposure to coherent light. No consistent differences are observed between the patched and the control eyes. Artefacts related to fixation and embedding are present in all tissues, however, these do not appear to be of sufficient degree to preclude this conclusion.

Sections prepared from animal LA-1 also contain artefacts related to fixation and embedding of tissues. These consist of prominent plastic tearing at the level of the ganglion cell nerve fiber layer, inner plexiform and inner nuclear layers. There is angulation of the outer segments and the interface between the retinal pigment epithelium and outer segments is distorted. There is considerable inter-disc separation within the outer segments of the rods and cones. These artefacts appear to be related primarily to imperfect infiltration of the tissue by plastic embedding material. Artefacts related to the length of time between enucleation and the fixative reaching the tissue are primarily manifested by cellular shrinkage, distortion, loss of fine detail, and intracellular vacuolization. Fewer artefacts are noted in the outer segments of the rods and cones, and in the retinal pigment epithelium than in the middle and inner portions of the retina. In some blocks from animal LA-1 sectioning was more difficult than in blocks N-403 and N-239. In a conference held at LAIR attended by Dr. Toichiro Kuwabara it was suggested that embedding artefacts can be reduced in the future by using epoxy resin instead of araldite.

Study of sections prepared from all blocks of tissues submitted to the contractor from the eyes of animal LA-1 do not, in our opinion, show ultrastructural evidence of tissue alteration that can be attributed to exposure to coherent light. No consistent differences are seen between corresponding areas of the retina of each eye. The artefacts noted above do not appear to be of sufficient degree to preclude this interpretation.

At a conference held with LAIR personnel and Dr. Toichiro Kuwabara, the photographs of the sections prepared by the contractor were compared with those prepared at LAIR and found to be comparable. The contractor was informed at that time that psychophysical studies had not shown spectral sensitivity differences between the treated and non-treated eyes prior to sacrifice of the animal. We have concluded that diffuse coherent light at the intensity and duration of exposure levels utilized by LAIR personnel has not produced ultrastructural alterations in the observed tissues.

Studies of normal turtle retina are currently in progress.

Recommendations

The absence of fine structural change in the exposed retinas at levels of coherent light exposure that in the past have been sufficient to cause longterm changes in spectral sensitivity suggests that the latter occurs as a result of functional, rather than morphologic alteration. The intensity and duration of exposure at the augmented levels used in animals LA-1 and the absence of associated ultrastructurally demonstrable injury suggests that further morphologic investigation along current lines will not be fruitful. Future investigations could be targeted at detecting enzyme histochemical abnormalities occurring in the percipient element outer segment, or in the retinal pigment epithelium of an appropriately exposed animal, and correlating the histochemical change with electro physiologic and ultrastructural details. The ongoing studies of normal turtle retina are being performed toward this aim.

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